PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Shigeru YAMAMOTO, et al.

Docket No: Q63731

Appln. No.: 09/806,413

Group Art Unit: 1652

Confirmation No.: 8678

Examiner: David J. Steadman

Filed: March 30, 2001

For: NOVEL ENZYME COMPOSITION AND PRODUCTION METHOD AND USE THEREOF

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Shigeru Yamamoto, hereby declare and state:

THAT I am a citizen of Japan;

THAT I graduated from Graduate School, Faculty of Pharmaceutical Sciences of Kanazawa University with a Master's Degree in March of 1993;

THAT I have been employed by Amano Pharmaceutical Co., Ltd. since March , 1993, where I hold a position a researcher with responsibility for research and development on enzymes, and their applications and genetic engineering in the field of foods;

THAT I am considered to be an expert in the field of the present invention, and that I am further fully knowledgeable of the disclosure of the present application and the materials contained therein; and

THAT I am one of the inventors of the instant patent application.

The present application discloses the discovery and characterization of a novel diglycosidase derived from the organism *Aspergillus fumigatus* that has the ability to cleave a disaccharide glycoside to yield saccharides in disaccharide units.

As discussed in detail at page 47, lines 8-10, of the specification, the molecular weight of the novel diglycosidase was determined to be 47 kDa by SDS-PAGE.

It is well known that there are a number of different methods that can be used to determine the molecular weight of a polypeptide. Because the different methods of measurement may use different criteria for calculating the molecular weight of a particular polypeptide, the skilled artisan would readily understand that different molecular weight values may be used to describe the same polypeptide.

For example, in addition to SDS-PAGE, the molecular weight of a polypeptide may also be determined based solely on the amino acid composition of the polypeptide through the use of computer software designed for such a purpose.

Such a computer program was used to calculate the molecular weight of the novel *Aspergillus fumigatus* diglycosidase of the present invention. Using the amino acid sequence set forth in SEQ ID NO:8 of the application, and the MacVector NTI DNA analyzing software (InforMax Co., Frederick, Maryland), the molecular weight of the novel diglycosidase was determined to be 50,891. A computer print-out of the data produced using the program is attached. The MacVector NTI DNA analyzing software used to perform the calculation has been recognized by more than 35,000 researchers world-wide as an appropriate program for determining molecular weight (see the attached brochure).

In view of the fact that the molecular weight of a polypeptide, such as the diglycosidase derived from *Aspergillus fumigatus*, may vary depending on the method used to calculate it, the skilled artisan would understand that the molecular weight of the diglycosidase could accurately be described as both having a molecular weight of "about 47 kDa," in view of the data from the SDS-PAGE analysis, and having a molecular weight of "about 51 kDa," in view of the data from computer software analysis.

Furthermore, due to factors such as glycosylation and tertiary structure, the skilled artisan would understand that the experimentally devised value of 47 kDa could be somewhat higher or lower. The molecular weight calculated by the computer software program was about 51 kDa. The skilled artisan would therefore consider the molecular weight of the novel diglycosidase of the present invention to be from "about 47 kDa to about 51 kDa."

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: October 7, 2003

Shigeru Yamamoto
Shigeru Yamamoto

Calculated Molecular Weight = 50891.33

Estimated pI = 5.29

Amino Acid Composition:

Non-polar:	No.	Percent
A	51	10.52
V	29	5.98
L	37	7.63
I	14	2.89
P	23	4.74
M	13	2.68
F	12	2.47
W	13	2.68

Polar:	No.	Percent
G	52	10.72
S	48	9.90
T	43	8.87
C	5	1.03
Y	25	5.15
N	36	7.42
Q	25	5.15

Acidic:	No.	Percent
D	22	4.54
E	6	1.24

Basic:	No.	Percent
K	16	3.30
R	4	0.82
H	8	1.65

Products Information

| TOP | Product list | Demo Soft Download | Data claim / E-mail distribution |

Vector NTI Suite 2 ver7 (Macintosh)

Vector NTI Advance (the win version) /// Vector Xpression (to discovery analysis) /// PathBlazer (to path way creation)

Various functions which need Vector NTI for arrangement analysis are substantial, and the ease of using and the graphical display are accepted in the researcher in about 35000 persons' world.
Moreover, by drag and drop, by the function, a file can be moved and it can develop.

Vector NTI Suite2 mainly consists of three modules of a VectorNTI basic module, AlignX, BioPlot, and ContigExpress.
Only the VectorNTI basic function is put on the market and Ver7 has become OSX correspondence now. In addition, about a function, Ver5.3 is used succeedingly, and immediately after becoming OSX correspondence, you can use. <

---

1. Vector NTI basic function
   - o Nucleic acid and amino acid arrangement are displayed graphically.
   - o An image, arrangement, the copy of a comment, an edit function
   - o Cloning simulation
   - o A molecular design and a parameter setup >
   - o Sequence registration, edit
   - o The additional display of a functional part
   - o A PCR plastic IMA design and analysis
   - o Sequence plastic IMA, high BURIDAIZESHON probe 設, and analysis
   - o A restriction enzyme part display, an ORF prediction display
   - o Electric 泳動 simulation
   - o Protein analysis function
     A molecular weight, amino acid composition, coordination 電点, a 吸光 coefficient, 吸光度, 荷電 calculation, etc.

2. Data management function
   - o Nucleic acid, protein, a restriction enzyme, an ORIGO nucleotide, a gel marker, a Blast result, etc. are group-ized, and preservation is possible.
   - o molecule Feature is imported from GeneBank and an EMBL file.
   - o Import export of data is possible.

o Th user field can be added according to a user's own data type.

3. Internet connection KUTIBITI interface
   - o Access to the database server of a public [VectorNIT] is possible.
   - o It is possible to send data directly for analysis. (An example, NCBI, BLAST search, etc.)
   - o A user can carry out file preservation of the information acquired from the Internet as his Molecule document automatically.
   - o Various analysis interfaces for AA for alignment, database reference, and BLOCKS and PROSITE reference and NA sequence analysis are prepared.

4. Align X - Multiple Sequence Alignment

   Align X attached to VectorNTI Suite It is the multiple alignment function which used ClustalW algorithm as the base.

   It uses, when considering the relation during the arrangement of two or more nucleic acid or two or more amino acid.
   - o Alignment is displayed graphically.
   - o Alignment editing function
   - o Evolution genealogical tree creation (the NJ method)
   - o Sequence comparison dot plot graph display function
   - o A sequence addition new to the existing result and analysis are possible.

5. BioPlot-arrangement analysis and protein analysis

   BioPlot is the software which performs arrangement analysis of nucleic acid or amino acid. In DNA/RNA analysis, energy. GC content, a melting temperature, etc. are analyzable.
   Moreover, in amino acid analysis, various analysis functions which made the start amino acid composition calculation, molecular weight calculation, parent water analysis, secondary structure prediction, etc. are attached.

6. ContigExpress-fragmentation ASSEN bull tool

   High-speed Assen BURESHON of DNA fragmentation is possible for ContigExpress of the VectorNTI Suite option. DNA auto sequencer The display of waveform data も is possible.

   Contig function
   - o The simultaneous display of waveform data and alignment
   - o It is ASSEMBURU operation, checking each data in a multiple

window.

- o The graphical display of a KONTIGU result
- o Preservation by GenBank, EMBL, and FASTA format is possible in a KONTIGU result.
- o Protein translation is possible.
- o Picture preservation of a waveform and a KONTIGU result image is possible at a camera function.

last updated:2003/04/01